

NaCl, KCl, NaNO<sub>3</sub>, NaHCO<sub>3</sub>, Na<sub>2</sub>SO<sub>4</sub>, Na<sub>2</sub>HPO<sub>4</sub>, NaH<sub>2</sub>PO<sub>4</sub>, NaNO<sub>3</sub>, KNO<sub>3</sub>, K<sub>2</sub>SO<sub>4</sub>, K<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, and KNO<sub>3</sub>.

**16.** The method of claim **15**, wherein one tonicifying agent is NaCl.

**17.** The method of claim **16**, wherein at the start of the incubation period the concentration of NaCl in the cell culture medium is 4.5 g/L or higher.

**18.** The method of claim **16**, wherein at the start of the incubation period the concentration of NaCl in the cell culture medium is 6.5 g/L or higher.

**19.** The method of claim **16**, wherein at the start of the incubation period the concentration of NaCl in the cell culture medium is 7 g/L or higher.

**20.** The method of claim **16**, wherein at the start of the incubation period the concentration of NaCl in the cell culture medium is 7.5 g/L or higher.

**21.** The method of any one of claims **14-20**, wherein at the start of the incubation period the cell culture medium contains an ionic tonicifying agent at a concentration sufficient to produce at least a 50% increase in total rAAV production and a 20% decrease in helper virus production compared to a host cell incubated in a medium with an osmolality of 266 mOsm/kg.

**22.** The method of claim **21**, wherein at the start of the incubation period the cell culture medium contains an ionic tonicifying agent at a concentration sufficient to produce at least a 100% increase in total rAAV production and a 30% decrease in helper virus production compared to a host cell incubated in a medium with an osmolality of 266 mOsm/kg.

**23.** The method of claim **22**, wherein at the start of the incubation period the cell culture medium contains an ionic tonicifying agent at a concentration sufficient to produce at least a 150% increase in total rAAV production and a 40% decrease in helper virus production compared to a host cell incubated in a medium with an osmolality of 266 mOsm/kg.

**24.** The method of claim **23**, wherein at the start of the incubation period the cell culture medium contains an ionic tonicifying agent at a concentration sufficient to produce at least a 200% increase in total rAAV production and a 50% decrease in helper virus production compared to a host cell incubated in a medium with an osmolality of 266 mOsm/kg.

**25.** The method of any one of claims **1-13**, wherein the tonicifying agent is a non-ionic tonicifying agent.

**26.** The method of claim **25**, wherein the tonicifying agent is a sugar.

**27.** The method of claim **26**, wherein the tonicifying agent is a disaccharide.

**28.** The method of claim **26**, wherein the tonicifying agent is selected from the group consisting of sucrose, fructose, glucose, galactose, mannose, maltose, and trehalose.

**29.** Then method of claim **28**, wherein the tonicifying agent is sucrose.

**30.** The method of claim **29**, wherein at the start of the incubation period the concentration of sucrose in the cell culture medium is 6.8 g/L or higher.

**31.** The method of claim **29**, wherein at the start of the incubation period the concentration of sucrose in the cell culture medium is 13.7 g/L or higher.

**32.** The method of claim **29**, wherein at the start of the incubation period the concentration of sucrose in the cell culture medium is 29.4 g/L or higher.

**33.** The method of claim **29**, wherein at the start of the incubation period the concentration of sucrose in the cell culture medium is 41.1 g/L or higher.

**34.** The method of any preceding claim, wherein the cell culture medium is a serum-free cell culture medium.

**35.** The method of any preceding claim, wherein the cell culture medium is a protein-free cell culture medium.

**36.** The method of any preceding claim, wherein the cell culture medium is selected from the group consisting of MEM, DMEM, RPMI, Ham's F-12 medium, Leibovitz's L-15 medium, and mixtures thereof, said medium being supplemented with one or more tonicifying agents.

**37.** The method of any preceding claim, wherein the cell culture medium consists essentially of DMEM supplemented with one or more tonicifying agents.

**38.** The method of any preceding claim, wherein the host cell is a mammalian cell.

**39.** The method of any preceding claim, wherein the host cell is selected from the group consisting of HeLa, HEK293, COS, A549, BHK, and Vero cells.

**40.** The method of claim **39**, wherein the host cell is a HeLa cell.

**41.** The method of any one of claims **1-37**, wherein the host cell is an insect cell.

**42.** The method of claim **41** wherein the host cell is selected from the group consisting of Sf9, Sf-21, Tn-368, and BTI-Tn-5B1-4 (High-Five) cells.

**43.** The method of any preceding claim, wherein the helper virus is selected from the group consisting of adenovirus, herpes virus, baculovirus, and recombinant forms of any of the foregoing viruses.

**44.** The method of any one of claims **1** to **40**, wherein the helper virus is an adenovirus (AV).

**45.** The method of claim **44** wherein the helper virus is Ad5.

**46.** The method of any preceding claim, wherein the host cell comprises a heterologous nucleotide sequence flanked by AAV inverted terminal repeats.

**47.** The method of any preceding claim, wherein the host cell comprises rep and cap genes.

**48.** The method of any preceding claim, wherein the host cell comprises helper virus genes.

**49.** The method of any preceding claim, wherein the host cell comprises a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, rep and cap genes, and helper virus genes.

**50.** The method of any preceding claim, wherein the host cell is incubated in the cell culture medium for a period of at least 2 days.

**51.** The method of claim **50**, wherein the incubation period is at least 3 days.

**52.** The method of claim **51**, wherein the incubation period is about 4 days.

**53.** The method of any preceding claim, further comprising the steps of harvesting and purifying the rAAV.

**54.** A cell culture system comprising:

a) a host cell capable of producing rAAV;

b) a helper virus; and

c) a cell culture medium with an osmolality of 360 mOsm/kg or higher when measured immediately after the host cell is introduced into the cell culture medium.

**55.** The cell culture system of claim **54**, wherein the cell culture medium has an osmolality of 375 mOsm/kg or higher.